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Winter survival strategies of cave-adapted amphipods in the Ice Caves of Sam's Point area of Minnewaska State Park Preserve, NY, USA

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Abstract

The Allegheny Cave Amphipod (*Stygobromus allegheniensis* Holsinger, 1967) is a troglobiotic crustacean native to caves in the northeastern United States, including the unique tectonic ice caves of New York's Shawangunk Ridge. These caves act as natural cold air traps, retaining ice even through summer, creating an extreme environment for aquatic cave-adapted stygobionts. A key question is how these amphipods survive winters when the caves freeze, encasing floors and walls in ice. While past lab studies suggested they can survive freezing, our research shows limitations: individuals fully encased in ice do not survive beyond four hours. However, when surrounded by ice but buffered by a thin layer of liquid water at 0.5 °C, amphipods can endure prolonged exposure to near-freezing temperatures. Field observations confirmed that live specimens persist in the liquid layer beneath the ice and remain active during winter, displaying movement and foraging for food in baited traps. Additionally, specimens varied widely in size, suggesting the presence of multiple reproductive cohorts rather than a single annual reproductive cycle triggered by spring thaw. These findings indicate that reproduction and mortality occur year-round and that adults are capable of surviving the ice cave's extreme winter conditions.

Keywords

Cryoprotectant, stygobite, troglobite

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Introduction

Aquatic invertebrates face significant challenges in environments where water freezes, especially in temperate and polar regions where seasonal ice formation can drastically alter their habitat. These organisms have evolved various strategies to survive freezing conditions, allowing them to endure periods of extreme cold and resume activity when conditions become more favorable (Storey and Storey 2005).

Cold temperatures may disrupt metabolic processes. As such, invertebrates may remain dormant (Storey and Storey 2004) until conditions improve. Freezing can cause lethal damage to cellular structures and dehydration. Some species have developed mechanisms to prevent ice formation within their bodies or tolerate intracellular freezing (Denlinger and Lee 2010). One of the primary survival strategies employed by organisms is the production of cryoprotectants, such as glycerol, which lowers the freezing point of bodily fluids and protects cells from ice formation (Fuller 2004).

Additionally, some species exhibit freeze tolerance, allowing ice to form in extracellular spaces while preventing it from entering cells, thus minimizing cellular damage (Storey and Storey 2011). In contrast, other species employ behavioral strategies, such as migration to deeper or warmer waters, to avoid freezing altogether. Aquatic crustaceans can use a combination of these tactics. One such example is *Branchinecta gaini*, the largest invertebrate in the Antarctic lakes (Benvenuto et al. 2015). The species inhabits lentic waters covered by ice and snow, but it can also survive for short periods completely encased in ice. In cases where specimens live in ephemeral pools, which dry or freeze and overwintering is not possible, resting eggs are employed. Resting eggs of the fairy shrimp *B. gaini* are still viable at –25 °C (Benvenuto et al. 2015).

While the ability of various taxa to survive in cold environments has been extensively documented, few cave-dwelling organisms have been studied for their tolerance to subzero temperatures or their ability to seek warmer microhabitats within caves (Issartel et al. 2006; Novak et al. 2014). Iepure (2018), after reviewing the handful of documented cave-adapted species inhabiting ice caves, mentioned only 12 species of crustaceans in the world, and concluded that "biospeleological surveys on caves with perennial or seasonal ice are still exceptional. The small number of studies reflects the perception that the subterranean habitats and environmental conditions of ice caves are too harsh to sustain terrestrial and aquatic populations."

The scarcity of reports may also be due to the general rarity of troglobitic species that experience subzero conditions in their cave habitats. In most cases, the temperature within caves closely mirrors the annual average surface temperature, with caves generally exhibiting a lower thermal amplitude than the surface (Medina et al. 2023). As a result, the extremely low temperatures of winter that occur on the surface rarely penetrate most caves. For instance, while cold limestone caves in alpine and pre-alpine regions typically maintain internal temperatures below 10 °C, they seldom reach freezing or subzero conditions (Lencioni et al. 2010). Caves function as natural temperature buffers, offering refuge to organisms during extreme climatic events (Medina et al. 2023). In high-latitude or high-altitude regions, while cave-adapted organisms face

challenges due to perpetual darkness, they rarely develop specific adaptations to cope with freezing temperatures, unlike their surface-dwelling counterparts.

One exception is the Allegheny Cave Amphipod (*Stygobromus allegheniensis* Holsinger, 1967), a fully depigmented, eyeless, and rather large (1.5 cm long) amphipod. The species is found in caves in Maryland, Pennsylvania, and New York. While most caves in its approximately 600 km long range (Holsinger 1967) never experience subzero temperatures, the Ice Caves of Sam's Point area of Minnewaska State Park Preserve, and Mohonk Preserves do. These caves are situated atop the Shawangunk Ridge in the mid-Hudson Valley (New York, USA). The topology of these caves is such that they act as cold air traps. Snow and cold air that enter the caves during the winter cannot escape. This refrigerated environment often preserves snow and ice into the summer. As they are aptly named, some of the Ice Caves' walls and floors are covered with solid ice (Fig. 1).

Previous studies, conducted in collaboration with the Nature Conservancy at the Sam's Point Area, and Mohonk Preserve, have demonstrated that during the summer and fall, cave streams and pools are inhabited by large populations of amphipods, sometimes numbering in the hundreds or even thousands (Espinasa and Cahill 2011). However, the fate of these populations during the winter and spring, when the Ice Caves of the Shawangunk Ridge experience subzero temperatures, remains largely unknown. To investigate this, Espinasa et al. (2015) subjected specimens to a temperature gradient in the laboratory, ranging from 0 °C to 21.5 °C. The results revealed that the amphipods exhibited a clear preference for temperatures around 14.4 °C. Most notably, while they displayed an immediate aversion to temperatures above 18.5 °C, they did not react as strongly to lower temperatures. Some specimens were observed walking on the ice in the cold section of the gradient, and one specimen even allowed itself to be encased in ice rather than swim toward warmer water. The study also found that specimens could survive being frozen in a block of ice for up to two hours (See video at www.youtube.com/watch?v=MgajTnWVl3s). Nonetheless, the authors remarked that they were unable to keep the amphipods alive when more than 2 hours in solid ice. Since the specimens they used had been collected on October, the authors proposed that gradual cooling in fall and winter might trigger metabolic changes, allowing cold acclimation through the buildup of cryoprotectants.

These laboratory findings suggest that the Allegheny Cave Amphipod may possess the capacity to endure the subfreezing conditions characteristic of its cave habitat. One objective of the present study was to assess whether individuals collected during mid-winter (January–February) exhibit greater tolerance to prolonged ice encasement compared to those collected in October. A second goal was to extend these laboratory observations to field conditions in order to explore several key hypotheses.

Foremost among these was the question of whether adult amphipods can be found within the caves during winter. If adults are absent, it could indicate a life history strategy similar to that of the Antarctic fairy shrimp *Branchinecta gaini*, which inhabits ephemeral pools that freeze solid in winter and relies on the viability of resting eggs to regenerate populations following the spring thaw. However, the hypothesis that *Stygobromus* resting eggs can independently survive freezing is complicated by

Figure 1. A the Ice Caves at Sam's Point act as natural cold-air traps, preserving snow and ice well into the summer **B** inside the caves, the walls and floors are coated with ice, presenting a challenge for aquatic stygobites.

amphipod reproductive biology: females carry fertilized eggs in a specialized brood pouch, or marsupium, where they are protected until hatching.

Therefore, the year-round presence of adult Stygobromus within the caves—and their spatial distribution and behavior—could offer important insight into their overwintering strategies. Specifically, whether they persist in a frozen state, enter dormancy or hibernation, or remain active beneath the ice within liquid water layers.

Materials and methods

Over the past decade, New York's winters have exhibited significant variability in temperature and snowfall, influenced by broader climatic patterns such as El Niño and La Niña. During the winter of 2024–2025, when the study took place, there was a return to more typical winter conditions, with temperatures closer to the long-term average. The overall seasonal average for winter 2024–2025 in New York City was 1.5 °C, which is about 0.8 °C below normal. There was also approximately 26.4 cm of precipitation recorded, which is below the historical average of 64.3 cm.

Two field trips were conducted to Sam's Point Ice Cave #1 (41°40'20"N, 74°20'47"W), located in Minnewaska State Park Preserve, NY, with permission from both Minnewaska State Park Preserve and the New York State Office of Parks,

Recreation, and Historic Preservation (Application # 2015-MIN-001). The first trip took place on January 26, 2025. The cave was accessed through its lower, main entrance, and 63 meters of its galleries were explored. Further exploration was halted by an ice wall. During this trip, the locations of ice and flowing water within the galleries were recorded. Temperatures were recorded with a hand-held thermometer or with a Seek Shotpro high-resolution thermal imaging camera. Approximately 30 meters from the main entrance there is a small pool (60 × 20 cm) where amphipod specimens are commonly observed during the summer and fall. On this occasion, however, the pool's surface was frozen. Using an ice axe, a hole was made to a depth of about 10 cm, revealing liquid water beneath. The water beneath the ice was approximately 12 cm deep. A bottle funnel trap, measuring 12 cm wide, 12 cm long, and with a 2 cm opening, was placed in the liquid water and baited with a piece of carrot (Norrocky and Hazelton 2010). The trap was left for 3 hours, after which its contents were examined for organisms. A single specimen of *S. allegheniensis* was found and returned to its natural setting.

A second field trip took place on February 2, 2025. This time, the cave was accessed via its upper entrance pit, requiring rope caving techniques for both descent and ascent. This entrance provides access to the deeper galleries of the cave. As with the first trip, the locations of ice and flowing water throughout the galleries were recorded. At the end of the cave, a 15-meter-long pool was found that was not covered by ice. A baited trap was placed in this pool and left for 24 hrs. The 14 specimens collected were transported alive to the laboratory. Body lengths (from the tip of the rostrum to the tip of the third uropod along a straight line) were measured from a photograph taken of the live individuals in a Petri dish.

Specimens were then individually placed in 5 cm diameter Petri dishes, each filled to a depth of 0.5 cm with cave water, and allowed to acclimatize for 24 hours at 2 °C. They were then subjected to -18 °C until 95% of the water was frozen (approximately 15 minutes). Afterward, the specimens were divided into four experimental conditions:

a) Three specimens were exposed to -2 °C for 3 hours, during which they were fully encased in ice.

b) Two specimens were exposed to -2 °C for 4 hours, with the specimens fully encased in ice.

c) Four specimens were exposed to -2 °C for 4.5 hours, with the specimens fully encased in ice.

d) Five specimens, after 95% of the water froze but leaving a thin film of liquid water around some parts of the body, were transferred to 0.5 °C. Under these conditions, the ice remained solid, but the film of water stayed liquid. Consequently, the specimens were only partially encased in ice throughout the experiment. The movement of body parts not encased in ice was monitored every 5 hours until the ice was thawed 27 hours later.

All the live specimens were then kept at 2 °C for three days when experiment was ended.

Results

Field observations

Exploration of the caves revealed a diverse range of microenvironments. Within Sam's Point Ice Cave #1, two distinct thermal zones were identified. The upper galleries were relatively warm and largely devoid of ice, with air temperatures ranging from 2.0 to 3.2 °C. Pools in this area remained unfrozen. As the descent continued toward the lower galleries, a distinct thermocline was encountered, marked by a drop in air temperature to between 0.4 and 1.2 °C, and a progressive buildup of ice along the walls and floor (Fig. 2A).

A key finding of this study is that, despite mid-winter conditions, the Sam's Point Ice Caves were not entirely frozen. Several areas contained liquid water, both in lentic pools and in small flowing streams (Fig. 2B). Notably, a large pool located at the far end of Sam's Point Ice Cave #1—approximately 15 meters in length and 2 meters deep—remained entirely free of surface ice during the visit, with a recorded water temperature of 6.6 °C. Given its depth, it is unlikely that this pool will freeze completely, even during colder winters.

At the time of exploration, the wall rock temperature was -1.0 °C in the coldest sections of the caves. The lower, colder galleries are accessed through narrow passages, which likely limit airflow and contribute to the formation of cold air traps. These confined galleries contain the greatest accumulation of ice. Despite the low ambient temperatures, liquid water—ranging from 1.7 to 3.3 °C—was still observed flowing into these colder areas, either emerging from fissures in the rock walls or seeping beneath the gravel substrate (Figs 3, 4).

In regions with the highest ice accumulation, most pools appear to be completely frozen (Fig. 5A). However, even in these ice-dense areas, holes made through the surface revealed liquid water beneath the ice (Fig. 5B). A baited trap placed into one of these holes (Fig. 5C) retrieved an amphipod.

Another baited trap left in the 15 m long, unfrozen pool found in the warmer section of the cave retrieved fourteen amphipods. The population exhibited a wide range of sizes (4.9, 6.1, 6.1, 6.2, 6.8, 7.0, 7.2, 7.4, 7.6, 7.7, 7.9, 8.2, 8.3, and 10.2 mm), where the smallest individual was less than half the size of the largest (Fig. 6).

Survival to freezing conditions in the laboratory

Our studies showed that when specimens are fully encased in ice, they survive for a limited amount of time – about a maximum of 4 hrs. Of the three specimens encased in ice for 3 hrs, 2 survived. Of the two encased in ice for 4 hrs, one survived. Of the four encased for 4.5 hrs, none survived. On the contrary, the five specimens that were allowed to have a small film of liquid water around their body, even when some parts of their body were partially encased in the ice, survived after 27 hrs in ice water (Figs 7, 8).

Figure 2. Diversity of Environments **A** in certain areas, thermoclines and cold air traps create conditions where the walls and floors of galleries are encased in ice. In contrast **B** other galleries contain pools and flowing streams that remain liquid throughout the winter.

Figure 3. Thermal imaging was used to measure temperatures within the cave. In the colder galleries, rock wall temperatures registered at -1.0 °C, while the surrounding air measured 0.4 °C. Notice liquid water was still observed flowing along the cave floor.

Figure 4. Recording the temperature in free-flowing water where **A, B** water was flowing out of cracks in the wall into the coldest galleries. Underneath the ice **C** water was also flowing through the gravel. The thermometer in the photos shows the temperature in degrees Fahrenheit.

Specimens showed full tolerance to conditions just above freezing. By the end of the experiment, those exposed to temperatures up to a maximum of 2 °C for a total of 123 hours—including 27 hours spent partially encased in ice—remained alive and actively moving. Specimens located beneath a sheet of ice but within a liquid water layer were observed swimming continuously. Under these conditions, amphipods were in constant contact with the ice while crawling, yet their behavior showed no signs of reduced activity (Fig. 9).

Discussion

The presence of *Stygobromus allegheniensis* within the Ice Caves of the Shawangunk Ridge is a notable ecological observation. Unlike most cave-adapted organisms in the Northeastern USA, which rarely experience subzero temperatures, the ice caves function as cold air traps, maintaining ice until summer. For an aquatic crustacean, surviving in freezing water presents a unique environmental challenge. Previous laboratory studies showed that these amphipods could crawl across ice, become encased by it, and survive being frozen (Espinasa et al. 2015). Likewise, the amphipod *Gammarus oceanicus*

Figure 5. Pools in the colder zones **A** often appear to be completely frozen during winter. However **B** a layer of liquid water can persist beneath the surface ice **C** A 10 cm-deep hole (red arrow) drilled into one such pool revealed liquid water flowing through the gravel beneath the ice. A baited trap (blue arrow) placed in the hole captured an amphipod, indicating that these organisms remain active during winter and can navigate the liquid phase in search of food.

from the arctic islands of Spitsbergen can survive being frozen into solid sea ice at a temperature of -6 to -7 °C (Aarset and Zachariassen 1988). These findings raised the possibility that *S. allegheniensis* could survive extended freezing periods, encased in ice, in a hibernation-like state.

Contrary to this assumption, in that same study (Espinasa et al. 2015) the authors noticed that the maximum amount of time amphipods could survive in a solid block of ice was 2 hours. Since the specimens were collected in October, the authors hypothesized that in the field, progressive cooling and freezing throughout the fall and winter could trigger a change in their metabolism. That would enable their body to adjust to long freezing conditions, lead to cold acclimation, and modify their crystallization temperature values by the accumulation of cryoprotective molecules such as glycerol and/or free amino acids. For this study, we tested the hypothesis of whether, unlike specimens collected in October, specimens collected in the middle of the winter (January-February), could survive for extended periods being encased in ice.

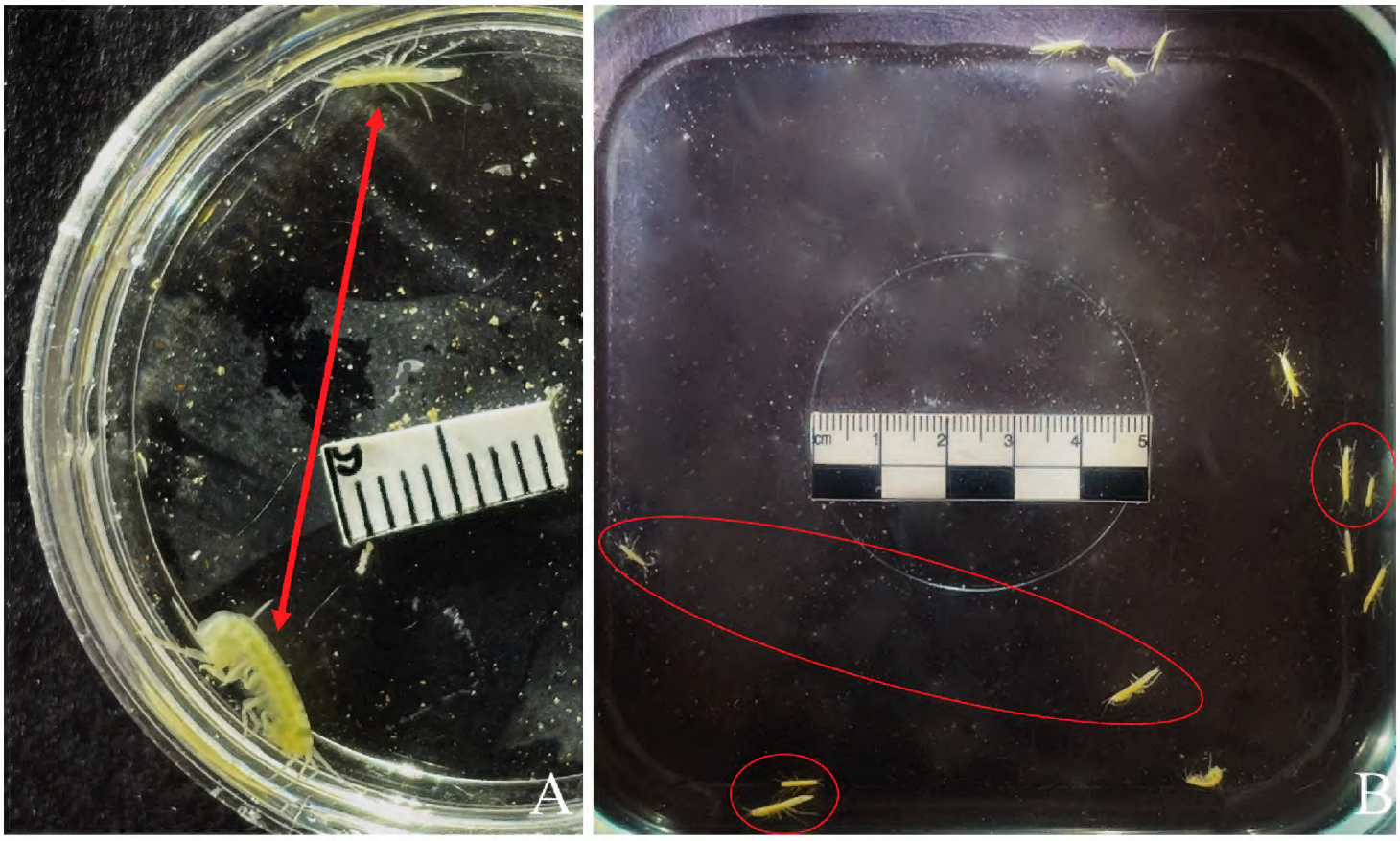

Figure 6. A extreme size difference between individuals **B** the considerable variation in size suggests that the population likely consists of individuals from multiple age groups. Circled in red are some of the examples of individuals with a wide variation in size.

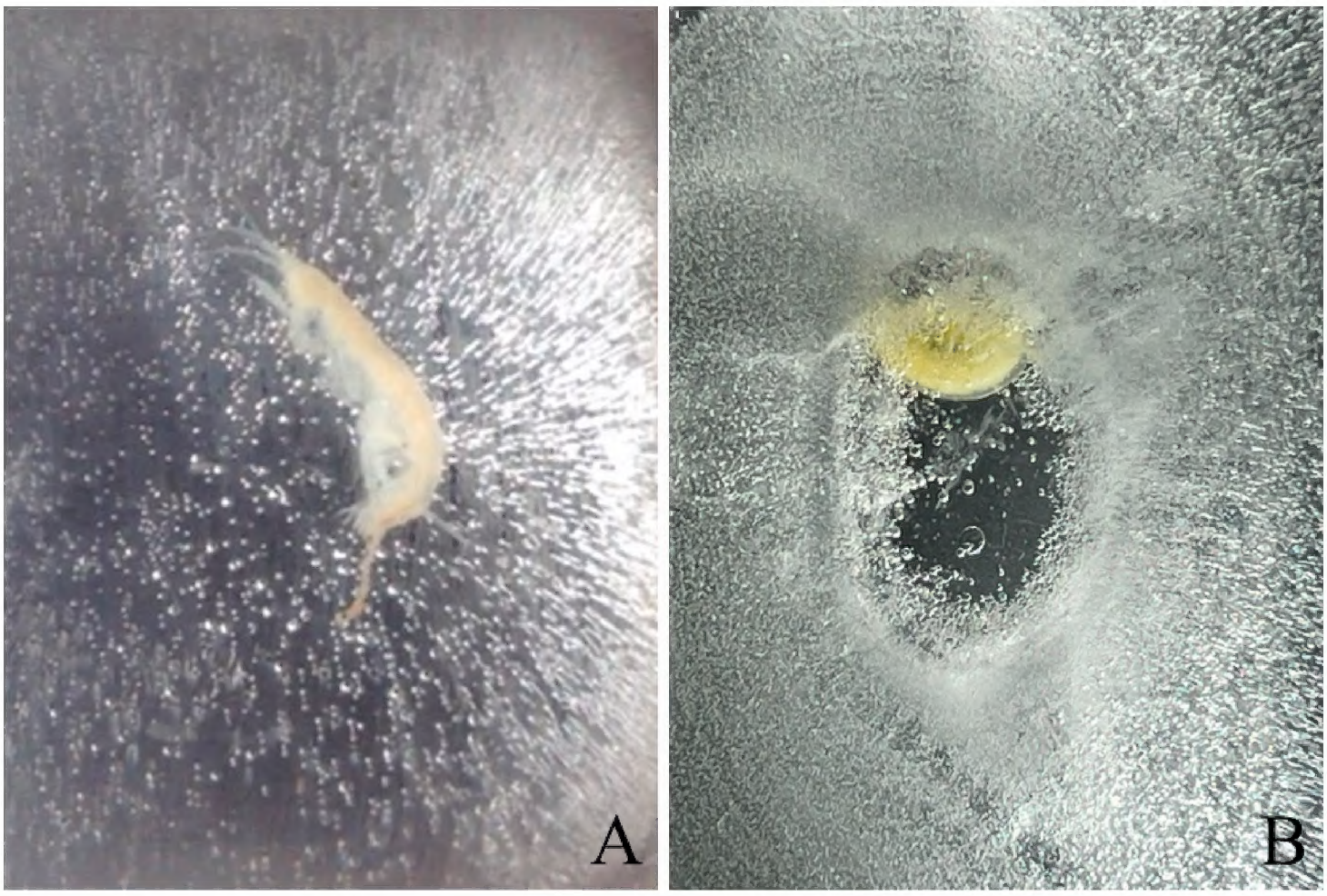

Figure 7. Survival in freezing conditions is highly dependent on the severity and duration of exposure **A** some individuals completely encased in ice at -2 °C for three to four hours were able to survive; however, none survived beyond 4.5 hours of exposure **B** in contrast, individuals only partially encased in ice at 0.5 °C were capable of surviving for at least 27 hours. Both individuals measure about 8 mm in length.

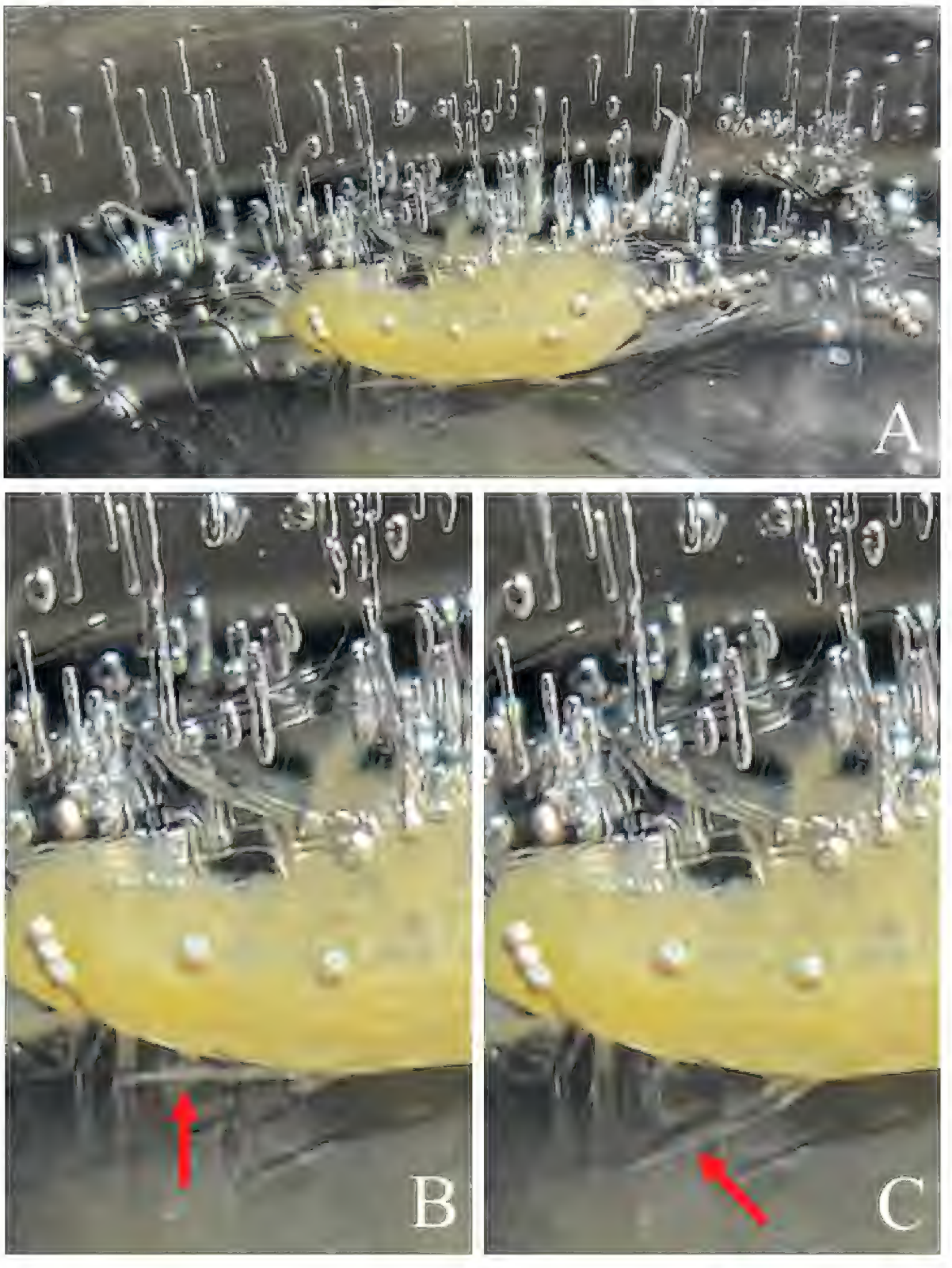

Figure 8. A specimen partially encased in ice and maintained at 0.5 °C **B, C** although most of the body was encased in ice, certain body parts—such as the leg (indicated by red arrows)—remained in contact with a thin film of water and exhibited movement. Remarkably, upon thawing after 27 hours, specimens kept under these conditions resumed active movement immediately. The specimen measured about 6 mm in length.

Our results did not support this hypothesis. While in our study survival was augmented to a maximum of four hours, it still falls short of being able to survive in a hibernation-like state, encased in ice, throughout the winter. While our study rejected that they have this extreme adaptation to freezing temperatures, our results provide the following conclusions as to how they survive the cold environment:

The *S. allegheniensis* population in the Ice Caves of the Shawangunk Ridge does not exhibit a life cycle in which all adult individuals perish during winter, with cold-resistant eggs generating a new generation after the spring thaw. A large variety of young

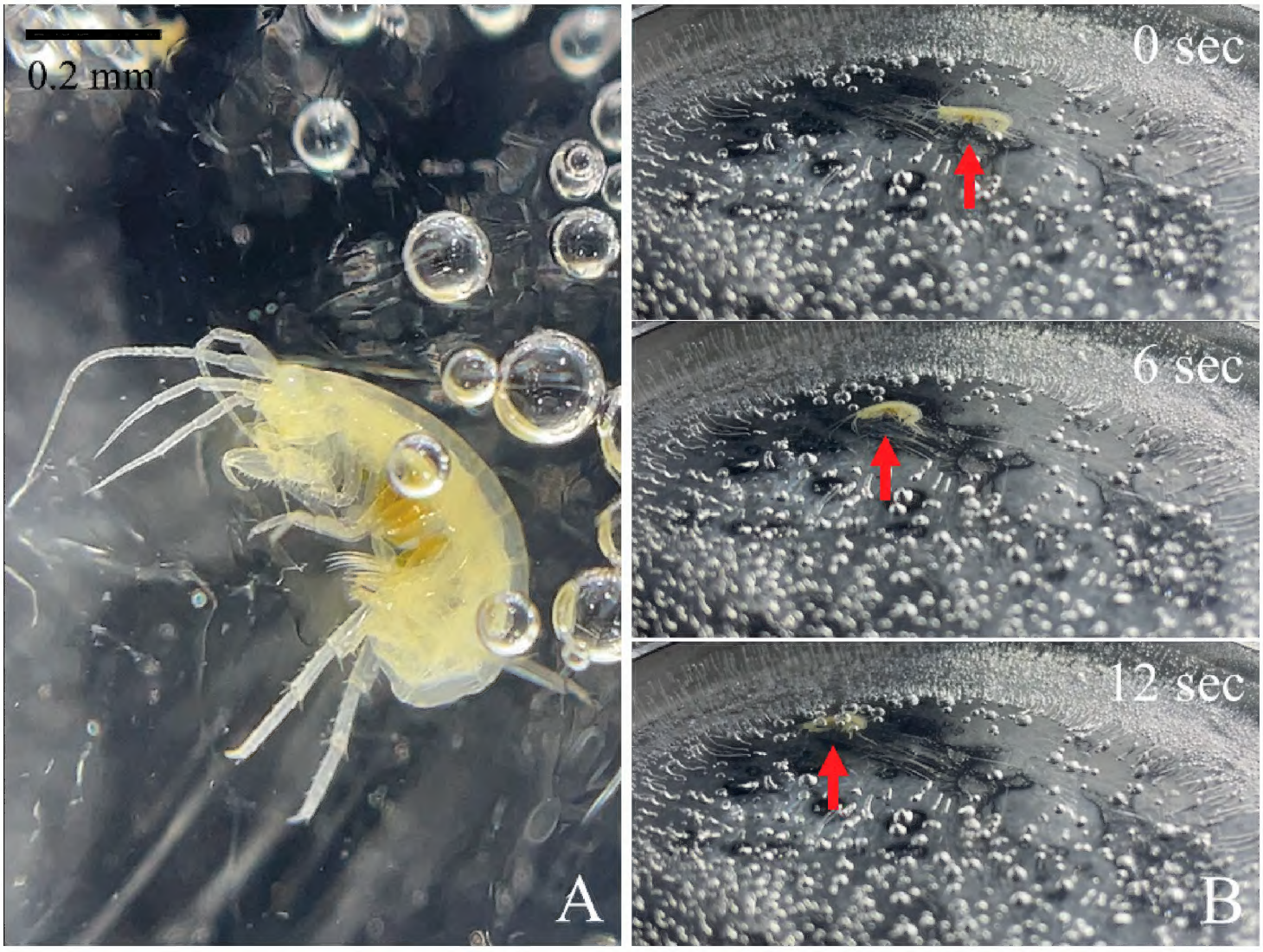

Figure 9. A amphipod swimming in the liquid layer underneath a sheet of ice **B** amphipods under these conditions remained active, being able to swim over twice their body length in 12 seconds.

and adult individuals were found cohabitating in the cave during winter. This suggests that the population in the Ice Caves consists of multiple reproductive cohorts, of different ages, which are alive throughout the winter. Size variation does not appear to be due to a sexually dimorphic trait. Holsinger (1978) reports that the maximum body length observed in *Stygobromus allegheniensis* is 13.5 mm for males and 13.0 mm for females. Thus, size of the species is not strongly tied to sexual dimorphism. The large size variability found in Ice Cave #1 (Stdev = 1.3 mm; Min 4.9 mm; Max 10.2 mm) suggests that the population is composed of differently aged individuals.

Individuals do not appear to undergo true hibernation, characterized by deep dormancy and complete inactivity. Animals in true hibernation, such as ground squirrels and certain bat species, enter a near-comatose state and rely on stored body fat to survive until the weather warms (Storey and Storey 2004). Instead, *S. allegheniensis* appears to engage in behavioral activities such as food-seeking behavior, as evidenced by being attracted to our baited traps. This suggests that some individuals maintain the ability to respond to stimuli (e.g., food odor from a carrot) and remain active enough to swim into a baited trap. This activity occurred in a shallow pool, whose surface was covered by a 10 cm layer of ice, indicating that the amphipods were still active despite near-freezing temperatures and potentially being in direct contact with the ice.

Our results suggest that some adult amphipods may survive prolonged periods surrounded by ice as long as there is a film of liquid water around their body. But there is another alternative. Espinasa et al. (2015) showed that *S. allegheniensis* is thermophilic towards temperatures around 14.4 °C. Our survey of the cave showed that even in the coldest portions of the cave, liquid water could be found. These cave amphipods are small enough to navigate through the gravel substrate, where liquid water continues to flow. This suggests that amphipods can avoid becoming trapped in ice by retreating into the warmer, free-flowing water within the gravel when the upper layers of the pool freeze. We suggest that the species may avoid near-freezing waters by seeking warmer microhabitats within the caves during winter. Our surveys of the cave revealed that many areas retain liquid water throughout the winter. It appears that most of the bedrock within the cave maintains temperatures close to the average surface temperature, which are above freezing, and only in pockets where cold air is trapped do surfaces exposed to the air experience subzero temperatures. This is supported by the presence of liquid water emerging from small cracks in the walls and seeping through the gravel during mid-winter. Given that *S. allegheniensis* seeks warmer water, it is likely that they can find refuge in microhabitats where water does not freeze, such as under gravel, in deep pools, or within cracks in the rock that shield them from the freezing air.

Adaptations to freezing conditions have been documented in another aquatic subterranean amphipod, *Niphargus rhenorhodanensis* (Issartel et al. 2006), from France. After inoculation at high subzero temperatures, cold-acclimated *N. rhenorhodanensis* survived. The accumulation of cryoprotective molecules such as glycerol (Issartel et al. 2006) and free amino acids (Colson-Proch 2009) may be linked to the survival of this species when this species was cold-acclimated.

That *N. rhenorhodanensis* has anti-freezing adaptations is paradoxical because the species does not inhabit caves that experience subzero temperatures. Issartel et al. (2006), assume that *N. rhenorhodanensis* survived the Quaternary glaciations at the edges or within nunataks—mountain peaks surrounded by ice but never covered by glaciers (Lefébure 2005). In these ancient environments, freshly melted water from the glacier, with temperatures near or just below 0 °C (Tweed et al. 2005), may have infiltrated the sediment and significantly influenced subterranean temperatures. As a result, the hypogean crustacean *N. rhenorhodanensis* could have encountered subzero temperatures and ice, potentially undergoing inoculative freezing.

Most of the caves in the range of *S. allegheniensis* also never experience below-zero temperatures. But just as with *N. rhenorhodanensis*, much of the range of *S. allegheniensis* was under the Laurentide Ice Sheet during the last glacial maximum, 20,000 years ago (Mickelson and Colgan 2003). It may be that the ancestor of all modern cave populations of *S. allegheniensis* experienced, and was adapted, to freezing conditions. These adaptations may have been the key to successfully colonizing the Ice Caves of Shawangunk Ridge post glaciation.

The results from this and previous studies suggest that *S. allegheniensis* may employ a combination of behavioral and physiological mechanisms to withstand the harsh winter conditions of the Ice Caves of the Shawangunk Ridge. Iepure (2018) review

on the ice caves fauna shows only 12 known species of crustaceans inhabiting such habitats world-wide. Given the scarcity of reports of cave fauna inhabiting this extreme environment, the findings of this study provide valuable insights into the survival strategies for extremely cold cave environments.

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References

Aarset AV, Zachariassen KE (1988) Low temperature tolerance and osmotic regulation in the amphipod *Gammarus oceanicus* from Spitsbergen waters. Polar Research 6(1): 35–41. https://doi.org/10.1111/j.1751-8369.1988.tb00579.x

Benvenuto C, Knott B, Weeks SC (2015) Crustaceans of extreme environments. In: Thiel and Watling. Lifestyles and Feeding Biology The Natural History of the Crustacea, Volume 2, Oxford University Press, 379–417.

Colson-Proch C, Renault D, Gravot A, Douady CJ, Hervant F (2009) Do current environmental conditions explain physiological and metabolic responses of subterranean crustaceans to cold? Journal of Experimental Biology 212(12): 1859–1868. https://doi.org/10.1242/jeb.047019

Denlinger DL, Lee Jr RE eds. (2010) Low temperature biology of insects. Cambridge University Press. https://doi.org/10.1017/cbo9780511675997

Espinasa L, McCahill A, Kavanagh A, Espinasa J, Scott A, Cahill A (2015) A troglobitic amphipod in the Ice Caves of the Shawangunk Ridge: Behavior and resistance to freezing. Subterranean Biology 15: 95–104. https://doi.org/10.3897/subtbiol.15.4733

Espinasa L, Cahill A (2011) Gigantism in Organisms from the Ice Caves at Sam's Point Preserve. The Northeast Natural History Conference. Albany, NY.

Fuller BJ (2004) Cryoprotectants: the essential antifreezes to protect life in the frozen state. CryoLetters 25(6): 375–388.

Holsinger JR (1967) Systematics, speciation, and distribution of the subterranean amphipod genus Stygonectes (Gammaridae). United States National Museum Bulletin 259: 1–176. https://doi.org/10.5479/si.03629236.259.1

Holsinger JR (1978) Systematics of the subterranean amphipod genus *Stygobromus* (Crangonyctidae): Part II. Species of the eastern United States. Smithsonian Contributions to Zoology 266: 266. https://doi.org/10.5479/si.00810282.266

Iepure S (2018) Ice cave fauna. In: Persoiu A, Lauritzen SE (Eds) Ice caves. Elsevier 2018: 163–171. https://doi.org/10.1016/b978-0-12-811739-2.00007-3

Issartel J, Voituron Y, Odagescu V, Baudot A, Guillot G, Ruaud JP, Renault D, Vernon P, Hervant F (2006) Freezing or supercooling: how does an aquatic subterranean crustacean survive exposures at subzero temperatures? Journal of Experimental Biology 209(17): 3469–3475. https://doi.org/10.1242/jeb.02387

Lefébure T (2005) Origine,évolution et mesure de la biodiversité des eaux souterraines: analyse moléculaire du genre Niphargus (Crustacea). PhD thesis, Université Claude Bernard, Lyon, France.

Lencioni V, Bernabò P, Latella L (2010) Cold resistance in two species of cave-dwelling beetles (Coleoptera: Cholevidae). Journal of Thermal Biology 35(7): 354–359. https://doi.org/10.1016/j.jtherbio.2010.07.004

Medina MJ, Antić D, Borges PA, Borko Š, Fišer C, Lauritzen SE, Martín JL, Oromí P, Pavlek M, Premate E, Puliafico KP (2023) Temperature variation in caves and its significance for subterranean ecosystems. Scientific reports 13(1): 20735. https://doi.org/10.1038/s41598-023-48014-7

Mickelson DM, Colgan PM (2003) The southern Laurentide ice sheet. Developments in Quaternary Sciences 1: 1–16. https://doi.org/10.1016/s1571-0866(03)01001-7

Norrocky MJ, Hazelton ER (2010) A Simple Device to Capture Amphipods (Malacostraca, Amphipoda). Crustaceana 83(3): 381–382. https://doi.org/10.1163/001121610X12627655658122

Novak T, Šajna N, Antolinc E, Lipovšek S, Devetak D, Janžekovič F (2014) Cold tolerance in terrestrial invertebrates inhabiting subterranean habitats. International Journal of Speleology 43(3): 3. https://doi.org/10.5038/1827-806x.43.3.3

Storey KB, Storey JM (2004) Metabolic rate depression in animals: transcriptional and translational controls. Biological Reviews 79(1): 207–233. https://doi.org/10.1017/s1464793103006195

Storey KB, Storey JM (2005) Freeze tolerance. Extremophiles. Eolss Publishers, Oxford, UK, 1–24.

Storey KB, Storey JM (2011) Molecular biology of freezing tolerance. Comprehensive Physiology 3(3): 1283–1308. https://doi.org/10.1002/cphy.c130007

Tweed FS, Roberts MJ, Russell AJ (2005) Hydrologic monitoring of supercooled meltwater from Icelandic glaciers. Quaternary Science Reviews 24: 2308–2318. https://doi.org/10.1016/j.quascirev.2004.11.020